Plasmid Extraction

Requirements:

—Omega E.Z.N.A.® Plasmid Mini Kit II
—100% ethanol
—Isopropanol
—Microcentrifuge capable of at least 13000 x g
—Nuclease-free 1.5mL microcentrifuge tubes
—Sterile deionized water
—Electric dry oven of 65°C
—Water bath of 65°C

Before Starting:

—Heat sterile deionized water to 65°C using water bath
—Add the vial of RNase A to the bottle of Solution I if there's no mark on the bottle and store at 4°C
—Add some 100% ethanol to the bottle of DNA Wash Buffer if there's no mark on the bottle and store at room temperature
—Add some isopropanol to the bottle of HBC Buffer if there's no mark on the bottle and store at room temperature
(The volume of ethanol and isopropanol is showed on the tag of the bottle)

Protocol:

1. Pellet 1.5mL bacteria in a clean 1.5mL microcentrifuge tube by centrifugation at 10,000 x g for 1 minute at room temperature. Decant or aspirate medium and discard.
2. Add 250µL Solution I/RNase, pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields,
3. Add 250µL Solution II and gently mix by inverting and rotating the tube several times to obtain a clear lysate. A 2 minutes incubation is necessary. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity.
4. Add 350µL Solution III and mix immediately by inverting the tube several times until a flocculent white precipitate forms. Incubate for 2 minutes.
5. Centrifuge at maximum speed (≥13,000 x g) for 10 minutes at room temperature. A compact white pellet will form. Promptly proceed to the next step.
6. Insert a HiBind® DNA Mini Column into a 2mL Collection Tube.
7. Transfer 700µL cleared lysate from Step 5 CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.
8. Centrifuge at maximum speed for 1 minute.
9. Discard the filtrate and reuse the collection tube.
10. Repeat Steps 7-9 until all cleared lysate has been transferred to the HiBind® DNA Mini Column.
11. Add 500µL HBC Buffer.
12. Centrifuge at maximum speed for 1 minute.

13. Discard the filtrate and reuse the collection tube.
14. Add 700µL DNA Wash Buffer.
15. Centrifuge at maximum speed for 1 minute.
16. Discard the filtrate and reuse the collection tube.
17. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.
18. Transfer HiBind® DNA Mini Column to a clean 1.5mL microcentrifuge tube. Open the lid and put it in the electric dry oven for 10 minutes to volatilize alcohol.
19. Add 60mL sterile deionized water directly to the center of the column membrane.
20. Let sit at room temperature for 2 minutes.
21. Centrifuge at maximum speed for 1 minute.
22. Store DNA at -20 °C